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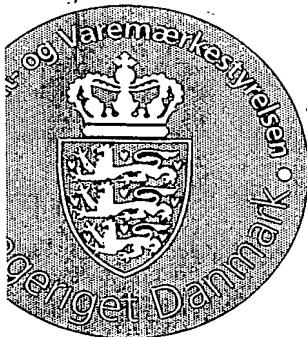
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Applicant:  
(Name and address)  
Novozymes A/S  
Krogshøjvej 36  
DK-2880 Bagsværd  
Denmark

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Pia Høybye-Olsen

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**FEED COMPOSITION AND METHOD OF FEEDING ANIMALS**

Modtaget

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**TECHNICAL FIELD**

PVS

- The present invention relates to a feed composition comprising hydrolysed fish protein and  
5 a method for feeding animals.

**BACKGROUND OF THE INVENTION**

Protein in the feed is essential for growing animals. To achieve the maximal growth rate protein should be available not only in the optimal amount but also in a form best suited for  
10 the uptake and utilisation by the growing animal. Fish protein is utilised in feed compositions for feeding many different kind of animals such as fish, pigs, poultry, cattle and fur animals, often in the form of fish meal. Fish meal is prepared from fish raw material by a heating, pressing, separation, and drying process, after which the product is in dried form known as fish meal. The nutritional value of fish meal is variable, depending on factors such as kind  
15 and freshness of the raw material and the processing conditions. To increase the nutritional value of feed compositions, the use of fish protein hydrolysate as a replacement for fish meal and other protein sources, has been proposed. Fish protein hydrolysate has a higher content of peptides and free amino acids, which should be easier digestible, especially to young animals whose digestive system is not fully developed. Fish protein hydrolysate  
20 additionally has the advantage that it can be produced from waste from fisheries and processing industries.

Fish protein hydrolysate can be in the form of fish silage produced by acid hydrolysis of fish protein. Espe et al. (Aquaculture 174 (1999) 119-137) discloses the partial replacement of  
25 fish meal by fish silage concentrate in a feed composition for Atlantic salmon. They observed a maximal growth rate when 15% of the fish meal was replaced by fish silage concentrate. With replacement of 25% or more of fish meal, they observed a lower growth rate than with no fish meal replacement.

- 30 Fish protein hydrolysate can also be prepared by enzymatic hydrolysis of fish protein, e.g. by the process disclosed in US 3697285 utilising an alkaline bacterial protease.

Oliva-Teles et al. (Aquaculture 179 (1999) 195-201) discloses the replacement of fish meal by enzymatically produced fish protein hydrolysate in a feed composition for turbot  
35 juveniles. They replaced 7%, 21%, and 35% of fish meal, respectively, in the feed

composition for 3 groups of fish, but found lower growth rates when replacing fish meal with fish protein hydrolysate than in a control group fed a feed composition without replacement.

- Cahu et al. (Aquaculture 171 (1999) 109-119) compared the growth rate of sea bass larvae fed with feed compositions where varying amounts of fish meal were replaced by enzymatically produced fish protein hydrolysate. They found the highest grow rate when no or 25% of fish meal was replaced, whereas the growth rate was significantly lower when 50% or 75% of fish meal was replaced by fish protein hydrolysate.
- 10 A fish protein hydrolysate that can replace substantial amounts of traditional protein sources in animal feed compositions with beneficial impact on animal growth rate is desired.

#### **SUMMARY OF THE INVENTION**

The present invention relates to a feed composition for feeding animals, prepared by a method comprising a) hydrolysing fish meat with a neutral protease and an alkaline protease, and b) inactivating the proteases by heat treatment.

In another aspect the invention relates to a method of feeding animals with a feed composition comprising a fish protein hydrolysate prepared by a method comprising: a) hydrolysing fish meat with a neutral protease and an alkaline protease; and b) inactivating the proteases by heat treatment.

#### **DETAILED DISCLOSURE OF THE INVENTION**

##### **25 Raw material for fish meat hydrolysis**

The fish meat used in the present invention may be any kind of fish material comprising protein. The fish meat may be whole fish bodies or parts thereof. Thus, the process of the invention may comprise a step of dividing whole fish bodies into pieces. The fish material may also be waste from filleting of red fish, e.g. salmon or trout, white fish, e.g. cod, haddock or pollack, tuna and mackerel. The fish material may be from any kind of fish, e.g. selected from the group consisting of white fish, red fish or pelagic fish, such as sprat, cod, haddock, tuna, sea eel, salmon, herring, sardine, mackerel, horse mackerel, saury, round herring, Alaska pollack, flatfish, anchovy, pilchard, blue whiting, trout, catfish, bass, capelin, Norway pout and hake or any combinations thereof. In a preferred embodiment the fish material comprises raw fish meat. By raw fish meat is meant fish meat that has not been subjected to temperatures above the denaturation temperature of the proteins, i.e. in one

embodiment above 70°C. In another embodiment of the invention the fish meat has been heated at above 80°C, preferably at temperatures between 90°C and 100°C, and may further have been pressed to remove oil. In one embodiment the fish meat is waste from fish oil production.

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#### Proteases

- A neutral protease according to the invention is defined as a protease with a pH optimum between 6.5 and 7.5, measured according to the Anson method as e.g. described in "Kinetic dimethyl casein method for determination of Alcalase activity by means of < Cobas > FARA centrifugal analyzer (SM-0218.02/01)" available upon request from Novozymes A/S, Bagsværd, Denmark. An alkaline protease according to the invention is defined as a protease with a pH optimum between 8 and 10, measured according to the Anson method. The neutral proteases and alkaline proteases may be of any origin, such as bacterial, fungal, vegetable or animal origin.

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- In a preferred embodiment of the invention the neutral protease is derived from a microorganism belonging to the species *Bacillus*, in a more preferred embodiment the neutral protease is derived from *Bacillus amyloliquefaciens*. In another preferred embodiment the neutral protease is derived from a microorganism belonging to the species *Aspergillus*, more preferably the neutral protease is derived from *Aspergillus oryzae*. An example of a commercially available neutral protease according to the invention is Neutraser™ (Novozymes A/S, Bagsværd, Denmark).

- In a preferred embodiment of the invention the alkaline protease is derived from a microorganism belonging to the species *Bacillus*, in a more preferred embodiment the alkaline protease is derived from *Bacillus licheniformis*. In another preferred embodiment the alkaline protease is derived from a microorganism belonging to the species *Aspergillus*, more preferably the alkaline protease is derived from *Aspergillus oryzae*. An example of a commercially available alkaline protease according to the invention is Alcalase™ (Novozymes A/S, Bagsværd, Denmark).

#### Hydrolysis

- According to the invention the fish meat is hydrolysed with a neutral protease and an alkaline protease. The hydrolysis may be conducted by any appropriate method known in the art. In a preferred embodiment the fish meat is mixed with water before hydrolysis. In one embodiment the amount of water is between 20% and 200% of the weight of the fish

- meat, such as between 40% and 150 %, especially between 75% and 125% of the weight of the fish meat. The hydrolysis may be carried out at any appropriate temperature. The temperature will usually be selected so as to achieve a high activity of the enzymes. In a preferred embodiment the temperature is below the denaturation temperature of the proteins, i.e. in one embodiment below approx. 70°C, such as e.g. between 20°C and 70°C, preferably between 40°C and 60°C, more preferably between 50°C and 60°C. The pH of hydrolysis may be any pH. In a preferred embodiment pH is selected so as to achieve a high activity of the enzymes. In another preferred embodiment pH is between 4 and 10, such as between 5 and 9, more preferably between 6 and 8. The pH may be the natural pH of the hydrolysis mixture or it may be adjusted by any methods known in the art such as addition of acid or base. The hydrolysis may be carried out for any time appropriate to achieve the desired result, such as between 10 minutes and 10 hours, preferably between 20 minutes and 4 hours, more preferably between 30 minutes and 2 hours.
- In a preferred embodiment the hydrolysis results in a degree of hydrolysis (DH) between 5% and 50%, such as between 10% and 30%, more preferably between 15% and 20%. DH may be determined by the OPA reaction method as described by Nielsen et al. Journal of Food Science 66 (2001) 642-646 and in "ED-9512723" available upon request from Novozymes A/S, Bagsværd, Denmark.
- Heat treatment**
- According to the invention hydrolysis is terminated by inactivating the proteases by heat treatment. The heat treatment may be carried out at any temperature and for any time sufficient to inactivate the proteases. In a preferred embodiment the heat treatment is carried out at 80-110°C for 10 seconds-1 hour, more preferably at 85-100°C for 5-30 minutes, most preferably at 90-95°C for 10-20 minutes.
- Removal of oil**
- In one embodiment of the invention the main part of the oil is removed after hydrolysis and inactivation of the enzymes to produce a substantially fat free hydrolysis product. The oil may be removed by any suitable method known in the art. In a preferred embodiment of the invention oil is removed by centrifugation in a separator or decanter centrifuge. In another preferred embodiment oil is removed in a 3-phase decanter further removing insoluble material from the water phase. By "substantially fat free" is meant that the main part of the oil is removed, leaving only the amount of oil usually left when using conventional methods known in the art for removal of oil. The amount of oil in the substantially fat free hydrolysis

product may be 0-5% (w/w) of the dry matter, such as 0-2% (w/w) of the dry matter, preferably 0-1% (w/w) of the dry matter.

- 5 In a preferred embodiment of the invention the hydrolysis mixture is passed through a sieve or filter before oil removal, to remove all or some of the insoluble material. In another preferred embodiment the hydrolysis mixture is passed through a filter after oil removal to decrease the turbidity of the product.

Concentration

- 10 In a preferred embodiment the hydrolysis product is concentrated by removal of water. Concentration may be performed by any method known in the art, such as evaporation or filtration. In a preferred embodiment the hydrolysis mixture is concentrated in a falling film evaporator and subsequently spray dried.
- 15 In one embodiment salt is added to the hydrolysate for preservation. In a preferred embodiment 5-25% (w/w) salt is added to the hydrolysate, such as 10-20% (w/w) salt, more preferably 10-15% (w/w) salt. Salt may be added at any appropriate point in the process, such as before, after, or during concentration.

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Feed composition

- In one aspect the invention relates to a feed composition prepared by a method comprising  
a) hydrolysing fish meat with a neutral protease and an alkaline protease; and b)  
inactivating the proteases by heat treatment. The term "feed composition" means any  
25 compound, preparation, mixture, or composition suitable for, or intended for intake by an animal. The feed composition may further comprise any other ingredient suitable for intake by animals, e.g. comprising sources of protein, lipids, carbohydrates, salts, minerals and vitamins. The ingredients may be selected, and mixed in any proportions, suitable to meet the nutritional needs of the animals to be fed with the feed composition and/or to keep the  
30 raw material cost of the feed composition within desired limits and/or to achieve other desired properties of the feed composition. Ingredients are usually selected among, but not limited to, the following materials: plant derived products, such as seeds, grains, leaves, roots, tubers, flowers, pods, husks, oil, soybean meal, soy protein isolate, potato protein powder, wheat, barley, corn, soybean oil, and corn gluten meal; animal derived products,  
35 such as fish meal, fish oil, milk powder, skim milk powder, bone extract, meat extract, blood

extract, and the like; additives, such as minerals, vitamins, aroma compounds, and feed enhancing enzymes.

The feed composition according to the invention may contain any amount of fish meat hydrolysate according to the invention suitable to meet the nutritional needs of the animals to be fed with the feed composition and/or to keep the raw material cost of the feed composition within desired limits and/or to achieve other desired properties of the feed composition. In a preferred embodiment the fish meat hydrolysate according to the invention constitutes 0.5-40% (w/w) of the dry matter of the feed composition, preferably 1-10 30% (w/w) of the dry matter of the feed composition, more preferably 2-16% (w/w) of the dry matter of the feed composition.

The feed composition may be prepared by any method known in the art, such as agglomeration, extrusion, expansion, or pelleting.

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#### Method of feeding animals

In one aspect the invention relates to a method for feeding animals with a feed composition according to the invention. The feed composition according to the invention may be in any suitable form and administered to the animals in any suitable way known in the art.

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#### Animals

The term "animals", as used herein, includes all animals. Examples of animals are non-ruminants, and ruminants such as cattle, sheep and horses. In a particular embodiment the animal is a non-ruminant animal. Non-ruminant animals include mono-gastric animals, e.g. pigs or swine (including, but not limited to, piglets, weaners, growing pigs, and sows); poultry such as turkeys and chicken (including but not limited to broiler chicks and layers); young calves; fish including, but not limited to, fresh water and salt water fish, fish larvae, growing fish, salmon, trout, turbot, sea bass, cod, pollack, sea bream, catfish, halibut, eel, carp; shrimp and sea urchin; and fur animals including, but not limited to, mink and fox.

30

### **EXAMPLES**

#### Fish protein hydrolysate

35 Fish protein hydrolysate according to the invention was produced from whole fish, heads, backbones and intestines from salmon, trout, pollack and herring, by the following process:

- Raw material and water were mixed in the ratio 1:1.
  - The mixture was heated in a reactor with agitation to 55°C
  - 1 kg Protamex™ (a mixture of a neutral protease and an alkaline protease available from Novozymes A/S, Bagsværd, Denmark) pr 1000 kg of raw material was added, and the reaction was followed by measuring pH, °Brix and Osmolality; the enzyme reaction was carried out for 45 minutes, followed by a heat inactivation of the enzyme at 95°C for 15 minutes.
- 5
- Sieving to remove bone material
- 10
- The mixture was separated using Westfalia SC35 and SB-7 separators. The mixture was separated into a liquid oil phase, a soluble protein fraction, and a paste of insoluble protein fraction.
  - The soluble protein fraction was evaporated using a Niro Atomizer FF 150 falling film evaporator.
- 15
- The evaporated concentrate was spray dried using a Niro Atomizer P-6 spray drier.

#### EXAMPLE 1

##### Replacement of fish meal by fish protein hydrolysate in a feed composition for salmon

- 20 50 fish (Atlantic salmon, approx. 125 g initial weight) were randomly allocated to each of 16 1 cubic meter tanks containing salt water at 7-8°C. Tanks were divided in 4 groups and each group was fed one of 4 experimental diets according to table 2. The diets were formulated to be iso-nitrogenous and iso-energetic on a crude protein and gross energy basis, and contained 10 % soy bean meal, approx. 14% starch from wheat, 35% lipid from  
25 fish oil and 35% crude protein. The 4 experimental diets contained varying amounts of LT-Fish meal (Norse LT-94 Norsildmel, Fyllingsdalen, Norway) and Fish Protein Hydrolysate prepared as described above. Composition of fish meal and fish protein hydrolysate is given in table 1
- 30 Uneaten feed was collected from the outlet water from the tanks as described by Helland et al., Aquaculture 139, 1996, 157-163. The fish were fed approx. 15% in excess based on expected feed requirements and uneaten feed in the spillage collectors. The fish were weighed individually with predetermined intervals during the experiment and fasted for one day prior to weighing.

**Table 1. Composition of fish meal and fish protein hydrolysate**

	LT-Fish meal	Fish Protein Hydrolysate
Dry matter (DM), g/kg	934.9	977.4
Lipid, g/kg DM	141.2	55.9
Starch, g/kg DM	3.1	0.7
Ash, g/kg DM	151.0	64.3
Crude protein, g/kg DM	752.2	902.9

Table 2. Formulation and composition of feed compositions, and growth data for salmon fed respective feed compositions (average  $\pm$  standard deviation).

	FM	FPH-05	FPH-10	FPH-15
<b>Formulation:</b>				
Fish meal <sup>1</sup> , g/kg	411.1	344.9	279.0	211.6
Fish protein hydrolysate, (FPH) g/kg	0.0	51.2	102.8	154.3
Fish oil, g/kg	313.0	320.1	327.5	334.4
Soybean meal <sup>2</sup> , g/kg	102.2	102.5	102.8	102.9
Wheat, g/kg	170.4	178.1	184.6	193.6
Constant ingredients <sup>3</sup> , g/kg	3.3	3.3	3.3	3.3
<b>Composition:</b>				
Dry matter, g/kg	948.7	945.0	946.8	943.7
Crude fish protein, g/kg	279	279	280	279
- from fish meal	279	234	189	143
- from FPH	0	45	91	136
% of crude fish protein from FPH	0	16	33	49
Gross energy, MJ	26.2	26.5	26.5	26.5
<b>Weight of fish fed the respective feed compositions:</b>				
0 days, g	163 $\pm$ 1	164 $\pm$ 1	163 $\pm$ 1	163 $\pm$ 1
28 days, g	210 $\pm$ 3	227 $\pm$ 1	231 $\pm$ 2	233 $\pm$ 2
56 days, g	285 $\pm$ 8	310 $\pm$ 7	325 $\pm$ 8	335 $\pm$ 5
<b>Specific growth rate<sup>4</sup> (SGR) of fish fed the respective feed compositions:</b>				
0-28 days	1.02 $\pm$ 0.06	1.29 $\pm$ 0.03	1.39 $\pm$ 0.02	1.43 $\pm$ 0.03
29-60 days	1.22 $\pm$ 0.06	1.25 $\pm$ 0.08	1.36 $\pm$ 0.07	1.45 $\pm$ 0.03

<sup>1</sup>Norse LT-94 (Norsildmel, Fyllingsdalen, Norway).

<sup>2</sup>Denosoy (Denofa, Fredrikstad, Norway).

<sup>3</sup>Constant ingredients, per kg: 2.9 g vitamin and mineral premix (G.O. Johnsen, Oslo, Norway); 0.3 g phosphorylated vitamin C (G.O. Johnsen); 0.1 g Y<sub>2</sub>O<sub>3</sub> (Sigma Chemical Company, St. Louis, Mo, USA).

<sup>4</sup>Specific growth rate: The percentual daily weight increase.

Results

Table 2 shows the average weight and specific growth rate of fish in the 4 different groups. It is seen that the growth rate increases with increasing substitution of fish meal with the fish protein hydrolysate of the invention. Surprisingly, the highest growth rate is obtained at the 5 highest level of substitution, where 49% (based on crude protein) of the fish meal has been replaced by the fish protein hydrolysate of the invention.

**EXAMPLE 2**

Growth of piglets when soy protein isolate is substituted with fish protein hydrolysate in the 10 feed composition.

48 piglets 24 days old and weighing 7.6 kg on average were distributed in 4 groups according to a randomised block design. The piglets were housed individually under standardised conditions. For 3 weeks the piglets were fed a prestarter feed mix and for the 15 subsequent 3 weeks a starter feed mix. Both were pelleted and offered for ad libitum intake. One group acted as control, in the 3 remaining groups varying amounts of soy protein isolate were substituted with the fish protein hydrolysate of the invention. Composition of the diets is given in table 1 and 2. Body weight gain, ad libitum feed intake and feed conversion ratio (g feed/g weight gain) were recorded.

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Table 1: Composition of the pre-starter diets (%)

Group	I	II	III	IV
Fish protein hydrolysate	-	2.0	4.0	6.0
Soy protein isolate	6.0	4.0	2.0	-
Skim milk powder	15.0	15.0	15.0	15.0
Potato protein powder	3.5	3.5	3.5	3.5
Corn gluten meal	2.76	2.85	2.92	3.00
Barley	20.0	20.0	20.0	20.0
Corn	20.0	20.0	20.0	20.0
Wheat	28.93	28.98	29.04	29.10
Soybean oil	0.74	0.69	0.63	0.57
L-Lysin · HCl	0.16	0.11	0.06	0.01
L-Tryptophan	-	-	0.01	0.02
Minerals and Vitamin premix	2.91	2.87	2.84	2.80

Table 2: Composition of starter diets (%)

Group	I	II	III	IV
Fish protein hydrolysate	-	2.0	4.0	6.0
Soy protein isolate	6.0	4.0	2.0	-
Potato protein powder	5.54	5.62	5.69	5.76
Barley	44.68	44.73	44.79	44.85
Corn	20.0	20.0	20.0	20.0
Wheat	20.0	20.0	20.0	20.0
Soybean oil	0.45	0.40	0.34	0.29
L-Lysin · HCl	0.26	0.20	0.15	0.09
L-Tryptophan	-	0.01	0.02	0.03
Minerals and Vitamin premix	3.07	3.04	3.01	2.98

Results

The average values of initial and final weights, growth rate and feed intake for the total experimental period are given in table 3. It is seen that the substitution of soy meal with fish protein hydrolysate according to the invention has beneficial influence on the growth of the piglets.

Table 3: Growth performance of piglets during the total experimental period (average ± standard deviation)

Group Fish protein hydrolysate (%)	I 0	II 2	III 4	IV 6
Initial weight (kg)	7.66 ±57	7.64 ±0.71	7.65 ±0.93	7.66 ±0.64
Final weight (kg) relative	27.45 ±2.80 100	27.83 ±2.71 101.4	28.15 ±3.51 102.6	28.34 ±1.36 103.2
Growth rate (g/day) relative	471 ±67 100	480 ±57 101.9	488 ±67 103.6	493 ±30 104.7
Feed intake (g/day) relative	732 ±94 100	743 ±87 101.5	760 ±104 103.8	767 ±50 104.8

**CLAIMS**

1. A feed composition for feeding animals, prepared by a method comprising:
  - 5 a) hydrolysing fish meat with a neutral protease and an alkaline protease, and  
b) inactivating the proteases by heat treatment.
  2. The feed composition of claim 1 further comprising removing the main part of the oil from the hydrolysed fish meat after step b).
  - 10 3. The feed composition of claim 1 wherein the animals are fish.
  4. The feed composition of claim 3 wherein the fish are selected from the group consisting of salmon, trout, turbot, sea bass, cod, pollack, sea bream, catfish, halibut, eel, carp.
  - 15 5. The feed composition of claim 1 wherein the animals are shrimps or sea urchins.
  6. The feed composition of claim 1 wherein the animals are pigs or swine.
  - 20 7. The feed composition of claim 1 wherein the neutral protease and/or the alkaline protease are derived from *Bacillus*.
  8. The feed composition of claim 1 wherein the neutral protease is derived from *Bacillus amyloliquefaciens*.
  - 25 9. The feed composition of claim 1 wherein the alkaline protease is derived from *Bacillus licheniformis*.
  10. The feed composition of claim 1 wherein the fish meat is raw.
  - 30 11. The feed composition of claim 1 wherein the fish meat has not been subjected to temperatures above 70°C before hydrolysis.
  12. The feed composition of claim 1 wherein the fish meat is hydrolysed to a degree of  
35 hydrolysis of 10-30%.

13. The feed composition of claim 1 wherein the fish meat is hydrolysed to a degree of hydrolysis of 15-20%.
14. A method of feeding animals with a feed composition comprising a fish protein hydrolysate prepared by a method comprising:
  - a) hydrolysing fish meat with a neutral protease and an alkaline protease, and
  - b) inactivating the proteases by heat treatment.